REMARKS

Claims 1, 15, 16 and 18-22 are pending in the subject application. By this Amendment, applicants have amended claim 1 to recite that the antisense oligonucleotide has the sequence of a human Ku70 cDNA or human Ku80 cDNA in the antisense orientation. Claim 15 has been amended to recite that antisense has the sequence of a human Ku70 cDNA in the antisense orientation. Support for both of these amendments can be found in the specification as originally filed at, inter alia, page 83, lines 7 to 16 and Fig. 13. Applicants maintain that the amendments to the claims raise no issue of new matter and respectfully request their entry. After entry of this Amendment, claims 1, 15, 16 and 18-22 will be pending and under examination.

Provisional Obviousness-Type Double Patenting Rejection

The Examiner provisionally rejected claims 1, 15, 16 and 18-22 under the judicially created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 27, 39 and 40 of copending U.S. Application No. 10/712,642.

In response, applicants respectfully traverse this obviousness-type double patenting rejection. Without conceding the correctness of the Examiner's position, applicants note that this is a provisional rejection over the U.S. Serial No. 10/712,642 which is not an allowed application. Accordingly, if the claims of the subject application are otherwise allowable,

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the provisional double patenting rejection should be withdrawn and the claims in the subject application should be allowed and issued, whereupon the claims of the U.S. Serial No. 10/712,642 could be assessed as to whether an obviousness-type double patenting rejection over a patent issued from the subject application would be warranted.

Rejections Under 35 U.S.C. §102(b)

The Examiner rejected claim 15 under 35 U.S.C. §102(b) as allegedly anticipated by Takiguchi et al. (Genomics, 35:129-135, 1996) for reasons as set forth in the previous Office Action.

In response, applicants respectfully traverse the Examiner's rejection. However, in order to expedite prosecution, and without conceding the correctness of the Examiner's position, applicants have herein amended claim 15 to recite that the claimed antisense oligonucleotide has the sequence of a human Ku70 cDNA in the antisense orientation. Takiguchi et al. does not teach such an antisense oligonucleotide. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Rejections Under 35 U.S.C. §103(a)

The Examiner rejected claim 15 as allegedly obvious over Takiguchi et al., as cited above, for reasons as set forth in the previous Office Action.

In response, applicants respectfully traverse the Examiner's rejection. However, in order to expedite prosecution, and without conceding the correctness of the Examiner's position, applicants have herein amended claim 15 to recite that the claimed antisense oligonucleotide has the sequence of a human Ku70 cDNA in the antisense orientation. Takiguchi et al. alone or in combination with ordinary skill, does not teach or suggest such an antisense oligonucleotide. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

The Examiner rejected claims 1, 15, 16 and 18-22 as allegedly obvious over Reeves et al. (J. Biol. Chem., Vol. 26499:5047-5052, 1989), Milner et al. (Nature Biotech. 15:537-541, 1997), and Takiguchi et al. (Genomics, 35:129-135, 1996) in view of AuYoung et al. (U.S. Patent No. 5,773,580) insofar as the claims are drawn to compositions and methods for increasing a target cell's sensitivity to DNA damaging agents in vitro comprising the administration of an antisense oligonucleotide specifically targeting a human DNA dependent protein kinase subunit.

In order for an obviousness rejection of the claimed method under 35 U.S.C. 103(a) to be proper, the prior art references, in combination, must in part teach or suggest all the elements of the claimed invention. Applicants note, however, that the cited references in combination do not teach or suggest an antisense oligonucleotide that specifically hybridizes to a nucleic acid encoding a human DNA-dependent protein kinase

subunit so as to prevent expression thereof, *wherein the antisense has the sequence of a human Ku70 cDNA in the antisense orientation* as recited in amended claims 1 and 15 or of a *human Ku80 cDNA in the antisense orientation* as recited in amended claim 1. In addition, the references in combination do not teach or suggest wherein such an antisense is enclosed in a liposome prior to introduction into the cell as set forth in amended claim 1.

In short, the cited references in combination do not teach or suggest all of the elements of the claimed invention. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Rejection Under 35 U.S.C. §112, First Paragraph (Written Description)

The Examiner rejected claims 1, 15, 16 and 18-24 under 35 U.S.C. §112, first paragraph, as allegedly not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner alleged that the claims do not adequately describe the distinguishing features or attributes shared by the members of the genus claimed.

In response, applicants respectfully traverse the Examiner's rejection. Applicants note that the composition and method

claims as amended encompass antisense oligonucleotides, or use thereof. The oligonucleotides specifically hybridize to a nucleic acid encoding a human Ku70 so as to prevent expression thereof. As such, the members of the genus need to possess all of the structural features determined from being (i) an antisense oligonucleotide (ii) that specifically hybridizes to a specific human nucleic acid encoding a human DNA-dependent protein kinase subunit, (iii) so as to prevent expression thereof, wherein (iv) the antisense oligonucleotide has the sequence of a human Ku70 cDNA in the antisense orientation or a human Ku80 cDNA in the antisense orientation. Thus, the members of the genus do not vary in the *requisite* structural features set forth in the claims and described in the specification. Furthermore, the human Ku70 gene sequence is known in the art. See Reeves et al. (1989), (**Exhibit 1**) and Genbank 51093847, (**Exhibit 2**).

Applicants maintain that those of skill in the *art of the claimed invention* would recognize from the description that the claimed antisense is described in the specification.

Thus, applicants maintain that the specification shows applicants were in possession of the claimed invention at the time of filing. Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Conclusion

For the reasons set forth above, applicants respectfully request that the Examiner reconsider and withdraw the rejections, and solicit allowance of pending claims 1, 15, 16 and 18-22.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee, other than the $510.00 extension fee, is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is required, authorization is given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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Molecular Cloning of cDNA Encoding the p70 (Ku) Lupus Autoantigen*

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The Ku (p70/p80) autoantigen consists of two phosphoproteins of molecular mass ~70,000 and 80,000 forming a macromolecular complex that binds DNA. Autoantibodies from a patient with systemic lupus erythematosus were used to isolate cDNA clones encoding the human ~70-kDa Ku antigen (p70) from a λgt11 expression library. The deduced amino acid sequence of p70 consisted of 609 amino acid residues and was confirmed by partial amino acid sequencing. The protein contains two acidic domains of 61 residues (31% Glu + Asp) and 19 residues (53% Glu + Asp) that are similar in size and charge to those found in a number of proteins involved in transcriptional activation. The 61-residue acidic region is rich in serine, raising the possibility that its charge might be modulated by phosphorylation. The predicted amino acid sequence also contains two regions with periodic repeats of either leucine alone, or leucine alternating with serine every seventh position. The latter repeat displays sequence and secondary structural similarities with the "leucine zipper" regions of the c-*myc* and v-*myc* oncogene products. The p70 antigen does not appear to have extensive sequence homology with the 80-kDa Ku autoantigen based on analysis of RNA blots and immunological criteria. A major antigenic determinant or determinants recognized by human autoantibodies is located near a leucine repeat on the carboxyl-terminal 190 amino acid residues of p70.

The p70/p80 autoantigen consists of two proteins of molecular mass ~70,000 and ~80,000 daltons that dimerize to form a 10 S DNA-binding complex (1). Exchange of immunological reagents has established that the p70/p80 antigen (1, 2), Ku antigen (3–5), Ki antigen (6), as well as a 86–70-kDa protein complex (7, 8)[1] are identical. The p70/p80 complex binds to the ends of double-stranded DNA (4) in a cell cycle-dependent manner, being associated with chromosomes of interphase cells, followed by complete dissociation from the condensing chromosomes in early prophase (2). Both p70 and p80 have been found to contain phosphoserine residues (8). The function of the antigen is unknown, but a role in DNA repair or transposition has been proposed (4, 5). Certain individuals with systemic lupus erythematosus (SLE)[2] and related disorders produce extremely large amounts of autoantibodies to p70 and p80 (1, 3, 6). We have used autoantibodies from the serum of an individual with SLE to isolate cDNA clones encoding p70, the protein that is thought to mediate binding of the Ku (p70/p80) complex to DNA (5). Analysis of the predicted amino acid sequence of p70 suggests structural similarities with other DNA-binding proteins. The amino acid sequence should be useful for examining the function of the Ku (p70/p80) complex, as well as the causes of autoimmunity to this antigen.

MATERIALS AND METHODS

Isolation of cDNA Clones—Human autoantibodies to the Ku (p70/p80) antigen from a patient (CK) with SLE were used to screen a human hepatoma λgt11 cDNA library, provided by M. Mueckler (Whitehead Institute, Cambridge, MA), using established protocols (9–11). Recombinant phage were plated on lawns of *Escherichia coli* Y1090 and overlaid with nitrocellulose filters (Schleicher & Schuell, BA85) impregnated with isopropylthiogalactoside (Sigma). Positive plaques were detected by incubating in blocking solution (150 mM NaCl, 50 mM Tris, pH 7.5, 1% bovine hemoglobin, 0.02% NaN_3) for 1 h at 22 °C, followed by CK serum (1:5000 in blocking solution, which was preadsorbed with bacterial lysate) (11) for 8 h at 4 °C, and ^{125}I-protein A (Du Pont-New England Nuclear; 10^6 dpm/ml) for 3 h at 22 °C. Three cDNA clones were obtained, the longest of which (~2.0 kb) was used to screen the same library by nucleic acid hybridization (12). Probes were labeled with [α^{32}P]dCTP by random priming (13) using Klenow fragment (Amersham Corp.). In addition, a 27-bp oligonucleotide 5′-CTTCCTCTGCTTCTTCATCGCCCTCGG-3′ complementary to the 5′ end of the of the 2.0-kb clone was synthesized (Applied Biosystems 380A DNA synthesizer), ^{32}P end-labeled with polynucleotide kinase (14) and used to rescreen the library (15).

Production of p70 Fusion Proteins—λgt11 clones 70.5, 70.34, and 70.77 were used to lysogenize *E. coli* Y1089, and fusion proteins were isolated as described (11). *E. coli* lysates containing the fusion proteins were analyzed on 8% SDS-polyacrylamide gels, and stained with Coomassie Brilliant Blue R250 (16).

Immunoblotting of the fusion proteins was performed as described (17). Blots were incubated in blocking solution for >1 h, followed by CK serum (1:250 dilution), or by the same dilution of CK serum plus an irrelevant autoimmune serum (patient JK) at a dilution of 1:250 for 3 h at 22 °C. After washing three times for 30 min, the blots were incubated with alkaline phosphatase-conjugated goat anti-human IgG antibodies (1:1500 dilution, from Tago, Burlingame, CA) for 3 h at 22 °C. Antibodies specific for the fusion proteins were purified by elution from the nitrocellulose blots (18) and used to probe immunoblots of K562 nuclear extract (2) followed by detection with ^{125}I-protein A as described above.

DNA Sequence Analysis—Restriction fragments of the phage cDNA inserts were subcloned into pUC 19, subsequently into

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J04611.

‡ Recipient of an Arthritis Investigator Award from the Arthritis Foundation. To whom correspondence should be addressed: the Rockefeller University, 1230 York Ave., New York, NY 10021.

[1] M. Yaneva, personal communication.

[2] The abbreviations used are: SLE, systemic lupus erythematosus; kb, kilobase(s); bp, base pair(s); SDS, sodium dodecyl sulfate.

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M13mp18 or M13mp19 (19), and sequenced from both strands by the dideoxy chain termination method (20). The rapid deletion subcloning technique of Dale *et al.* (21) was utilized to generate a sequential series of overlapping clones for sequencing. Oligonucleotides were synthesized and used without further purification (22) as primers for sequencing certain large fragments. Modified T7 DNA polymerase (Sequenase, United States Biochemical Corp., Cleveland, OH) using dITP in place of dGTP (23) was used for dideoxy sequencing of DNA regions not adequately resolved with Klenow fragment.

Computer Sequence Analysis—Sequences were assembled and analyzed by computer programs provided by the BIONET National Computer Resource for Molecular Biology. The translated amino acid sequence of p70 was compared to sequences in the National Biomedical Research Foundation Protein Identification Resource (PIR) using the algorithms of Lipman and Pearson (24, 25). Statistical significance of alignments was evaluated using the RDF program (24).

Protein Sequencing—Ku (p70/p80) antigen was purified from ~3.5 × 10^9 K562 cells as described.[3] Protein A-Sepharose beads were coated with monoclonal antibody 162 (1) at 4 °C for 8 h, washed three times with 150 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, 0.5% Nonidet P-40, 1 mg/ml ovalbumin, 0.02% NaN_3, and added to an extract of K562 cells (in 150 mM NaCl, 50 mM Tris, pH 7.5, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride) for 3 h at 4 °C. The beads were washed three times with 150 mM NaCl, 50 mM Tris, pH 7.5, 2 mM EDTA, 0.25 M sucrose, 2.5% Triton X-100, 0.5% SDS, then three times with 150 mM NaCl, 50 mM Tris, pH 7.5, 2 mM EDTA, and heated to 100 °C for 3 min in SDS sample buffer (16) before resolving on 10% SDS-polyacrylamide gels. The gels were stained with Coomassie Brilliant Blue R-250, and gel slices containing p70 were excised. The protein was electroeluted from the gel exactly as described by Hunkapiller *et al.* (27).

Electroeluted p70 was cleaved with chymotrypsin (Worthington) as follows: approximately 7 μg of p70 in 60 μl of 0.125 M Tris, pH 6.8, 0.5% SDS, 10% glycerol, 0.0001% bromphenol blue was heated to 100 °C for 3 min before adding chymotrypsin to a final concentration of 17 μg/ml. The sample was incubated for 30 min at 37 °C; digestion was terminated by the addition of SDS to 2.5% and dithiothreitol to 0.1 M. The sample was then heated to 55 °C for 10 min and loaded onto a 12.5% SDS-polyacrylamide gel.

After electrophoresis, intact p70 and chymotryptic peptides were transferred to polyvinylidene difluoride membrane (Immobilon, Whatman, Clifton, NJ) (28). After visualization by Coomassie Blue staining, p70 and p70 peptides of ~29, 22, and 16 kDa were excised from the blot and subjected to automated Edman degradation with the Applied Biosystems model 470A gas-phase sequencer. The phenylhydantoin amino acid derivatives were identified and quantitated using a Hewlett Packard 1084 HPLC system.

RNA Blot Analysis—K562 poly(A)$^+$ RNA (29, 30) was separated on 0.8% agarose gels containing 2.2 M formaldehyde (14), transferred to nitrocellulose, and baked for 90 min at 80 °C (31). DNA probes were labeled by random priming (13) as described above. RNA blots were prehybridized for 6–12 h at 42 °C in 5 × SSPE (1 × SSPE = 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM EDTA), 10 × Denhardt's solution (1 × = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 50% formamide, 0.4 mg/ml denatured sonicated salmon sperm DNA, 0.1% SDS before hybridizing for 30 h in the same solution containing probe at 10^6 dpm/ml at 42 °C. The blots were washed at 65 °C with 2 × SSC (1 × SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.4), 0.1% SDS (three times, 10 min each) followed by 0.3 × SSC, 0.1% SDS (three times, 45 min each), and exposed to X-ray film (XAR-5, Kodak, Rochester, NY) with Lightening Plus intensifying screens (Du Pont-New England Nuclear).

RESULTS

Isolation of cDNA Clones Encoding p70 Epitopes—A λgt11 expression library was screened with serum from a patient (CK) with high titer anti-Ku (p70/p80) antibodies. This serum contains anti-Ku (p70/p80) antibodies at a titer of approximately 1:3 × 10^6, along with low levels (1:1000 titer or less) of anti-RNP and anti-Sm antibodies (32). At the 1:5000 dilution used for screening, the serum was essentially monospecific for p70. Screening the λgt11 library with this serum yielded three positive plaques, designated clones 70.5, 70.34, and 70.77, respectively (Fig. 1). After plaque purification, *Eco*RI digestion of purified phage DNA demonstrated insert DNA fragments of approximately 1600 and 350 bp (clone 70.5), 900 bp (clone 70.34), and 700 bp (clone 70.77). On Southern blots, insert DNA from clone 70.77 hybridized with insert DNA from clone 70.34, and with the ~1600-bp fragment from clone 70.5 (not shown). DNA sequence analysis (see below) confirmed that the three clones contained fragments of the same gene.

Nucleic acid hybridization screening yielded additional λgt11 clones hybridizing with both the clone 70.77 insert and with the ~350-bp fragment of clone 70.5. Restriction mapping suggested that two of these clones, designated 70.30 and 70.45 (Fig. 1) contained additional DNA sequences not contained by clone 70.5. Screening with the 5′-oligonucleotide failed to yield clones with longer inserts.

E. coli lysogenic for λgt11 clones 70.34 and 70.77 produced fusion proteins of ~145 and ~140 kDa, respectively, after induction with isopropylthiogalactoside (Fig. 2). *E. coli* lysogenic for clone 70.5 produced only trace quantities of fusion protein (not shown). Autoantibodies from CK serum were affinity purified on nitrocellulose-bound 70.34 or 70.77 fusion proteins and used to probe immunoblots of total nuclear proteins (Fig. 3). The affinity-purified anti-70.34 and anti-70.77 antibodies specifically bound to p70 on immunoblots of total nuclear proteins, while autoantibodies in the original CK serum bound to both p70 and p80 (Fig. 3*A*). Addition of JK autoimmune serum to CK serum resulted in binding to additional proteins on immunoblots (Fig. 3*B*, *CK+JK*). The contaminating JK autoantibodies were removed by affinity purification on 70.34 and 70.77 (Fig. 3*B*), demonstrating the specificity of binding to the fusion proteins.

DNA Sequence—The nucleotide sequence of cDNAs from clones 70.5, 70.34, 70.77, 70.30, and 70.45 was determined from both strands using the sequencing strategy shown in Fig. 1. The nucleotide sequence (Fig. 4) contains a single open reading frame of 1,827 bp (from nucleotide 34 to 1,860), coding for 609 amino acids. The predicted molecular mass of the encoded p70 protein is 69,851, in close agreement with the apparent molecular mass of 70,000 estimated by SDS-polyacrylamide gel electrophoresis (1). The open reading frame is preceded by a 5′-untranslated region of 33 bp, and followed by a 3′-untranslated region of 294 bp terminating with a

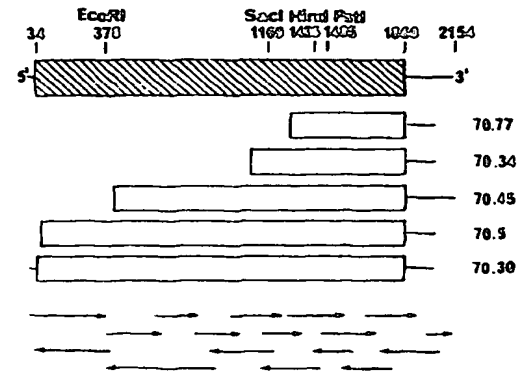

FIG. 1. **p70 partial restriction map, clones, and sequencing strategy.** The coding region (bases 34–1860) is shown as a *hatched box* in the partial restriction map (*top*). The individual cDNA clones obtained by screening with antibody probes are labeled *70.77* (bases 1286–2027), *70.34* (bases 1112–2025), and *70.5* (bases 44–2021). Additional cDNA clones obtained by nucleic acid hybridization are labeled *70.45* and *70.30*. The sequencing strategy is indicated by *arrows* at the *bottom*.

[3] W. H. Reeves, Z. M. Sthoeger, and R. G. Lahita, manuscript submitted for publication.

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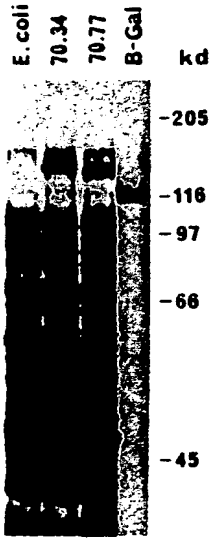

FIG. 2. **SDS-polyacrylamide gel of fusion proteins obtained from *E. coli* Y1089 lysogenized by λgt11 clones.** *E. coli* were solubilized in SDS sample buffer, and proteins were resolved on an 8% SDS-polyacrylamide gel followed by Coomassie Blue staining. Lanes show *E. coli* Y1089 lysate, and lysates of *E. coli* Y1089 lysogenized by clones 70.34 and 70.77. The last lane shows purified β-galactosidase (Sigma) for comparison. Positions of molecular mass markers are indicated on the *right*. *kd*, kilodaltons.

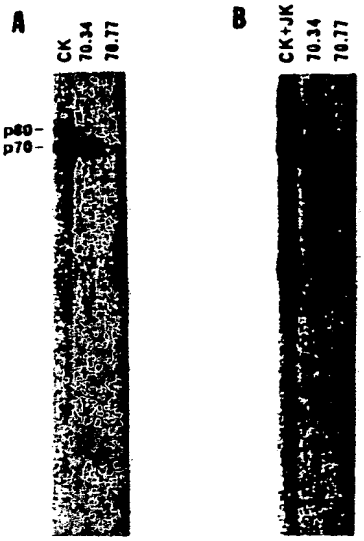

FIG. 3. **Immunoblots of antibodies affinity-purified from blots of fusion proteins.** *A*, immunoblots of K562 nuclear extract using CK serum (1:500) or CK antibodies (initial serum dilution 1:250) affinity-purified from 70.34 or 70.77 fusion proteins, respectively. On immunoblots of total nuclear extract, CK serum reacted with both p70 and p80, while the affinity-purified antibodies were specific for p70. *B*, immunoblots of K562 nuclear extract using CK plus JK sera (both at 1:500 dilution) or CK plus JK sera (initially each at 1:250) affinity-purified from 70.34 or 70.77 fusion proteins, respectively.

AATAAA sequence followed by a 68-bp poly(A) sequence. Two clones (70.5 and 70.44) had a cytidine at position 300, while two others (70.30 and 70.26) had a thymidine. The substitution does not change the predicted amino acid sequence and may represent allelic variation.

The sequence AACATG (nucleotides 31–36) is a potential ribosome binding site (33) which may encode the initiator methionine as indicated in Fig. 4. However, this prediction could not be confirmed by amino acid sequencing because the amino terminus of p70 was blocked.

Partial Amino Acid Sequence of p70—Since the amino-terminal sequence of p70 was unobtainable, the protein was cleaved with chymotrypsin and partial amino acid sequences of peptides of molecular mass ~29, 27, and 16 kDa were determined. The amino acid sequences of the three peptides match the predicted amino acid sequence as shown in Fig. 4 (*single letter code*), confirming the identity of the cDNA clone.

RNA Blot Analysis—Probes consisting of the 3′ ~1640 bp and 5′ ~340 bp of clone 70.5 each hybridized with a single mRNA species of ~2.4 kb (Fig. 5, *probes A* and *B*, respectively). Thus, although the entire coding sequence has probably been determined, the sequence of the 5′-untranslated region is likely incomplete.

p70 Has a Cluster of Acidic Amino Acids and Periodic Repeats of Leucine or Leucine and Serine Residues—Examination of the predicted amino acid sequence of p70 revealed the existence of a high concentration of negatively charged residues near the amino terminus. The first 61 amino acids consist of 31% glutamic acid + aspartic acid, with a 19-amino acid region (residues 10–28, *underlined* in Fig. 4) consisting of 58% Glu + Asp. In addition, the amino-terminal 81 amino acids contains 13 serine residues (16%). A shorter acidic domain is present from residues 328–340 (7/13 residues or 53% Glu + Asp, *underlined* in Fig. 4).

Comparison of the amino acid sequence with known sequences in the National Biomedical Research Foundation Protein Identification Resource database revealed a possibly significant similarity with the v-*myc* oncogene product (Fig. 6). A region of p70 from amino acid 187 to 248 (62 residues) was 27% identical with a region of the v-*myc* oncogene protein from amino acid 361 to 422, and displayed weaker similarity with the c-*myc* protein. Statistical analysis of this alignment using the RDF program (24) gave an initial score of 62 (z = 9.59 S.D.) the aligned score of 62 (z = 5.62 S.D.). This region of both v-*myc* and c-*myc* contains a "leucine zipper" domain characterized by the periodic repetition of leucine residues every seventh position in an α-helical region (34). The p70 sequence has identical periodicity, but instead of having leucine residues at every seventh position, has leucine alternating with serine (Figs. 4, 6, and 7, indicated by *). Secondary structure predictions for p70, v-*myc*, and c-*myc* in this region are suggestive of α-helix formation (Fig. 7). Immediately adjacent to this region (toward the carboxyl terminus) is a 22-amino acid region containing 50% basic residues (Fig. 7, indicated by x), as appears in other proteins with leucine repeats (34). Another possible leucine repeat in p70 occurs from amino acids 483 to 511 (Fig. 4, residues at seventh positions indicated by *), but contains a proline residue (residue 500) that might destabilize a region of α-helix.

DISCUSSION

The Ku (p70/p80) antigen is recognized by autoantibodies in sera of certain patients with SLE (1) and other (3) collagen vascular diseases. The function of this antigen is not known, but previous studies have shown that the p70 and p80 proteins form a complex (1, 6, 7) that binds to DNA (1, 4, 5, 7). Binding to DNA may be mediated by p70 (5) and also be specific for ends of double-stranded DNA, suggesting a possible role in DNA repair or transposition (4).

These previous studies suggest that the p70 protein contains a region, or regions, mediating binding to DNA and to p80. As a first step to defining these regions, we have cloned and sequenced cDNA encoding p70. The translated amino acid sequence consists of 609 amino acids (Fig. 4). However, the

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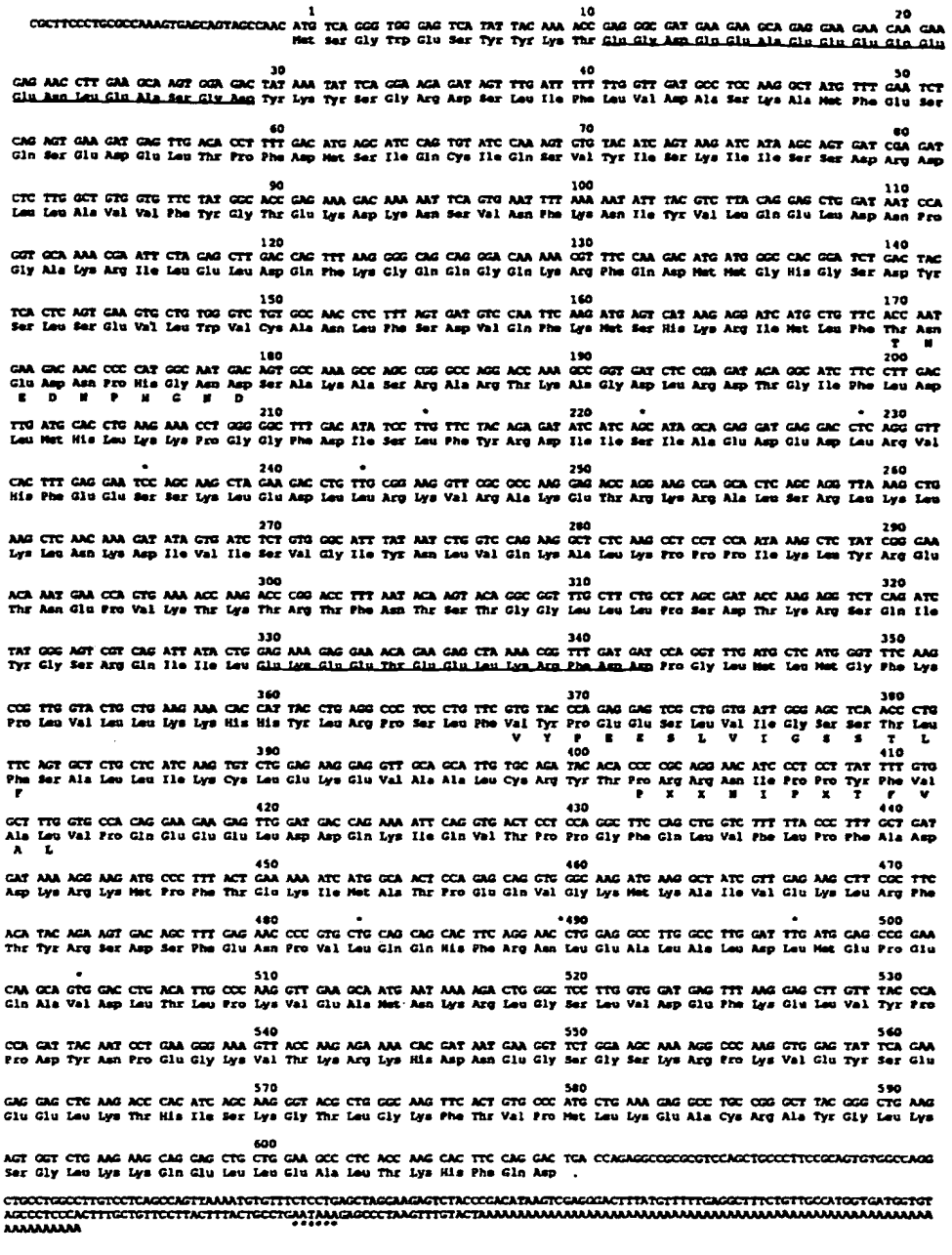

FIG. 4. **Nucleotide and translated amino acid sequence of p70.** DNA sequence is shown *above*, and predicted amino acid sequence *below* in *three-letter code*. Numbering corresponds to the predicted amino acid sequence. Amino acid sequences determined by automated Edmann degradation are indicated by *one-letter code* beneath the predicted amino acid sequence. Anionic domains of the translated protein (residues 11–29 and 330–342) are *underlined*. Periodic repeats of leucine and/or serine residues are indicated by *. A potential polyadenylation signal (AATAAA) is indicated (.....).

predicted initial methionine may be cleaved *in vivo*, since it is followed by serine, a residue that promotes removal of amino-terminal methionine residues by an amino-terminal methionine aminopeptidase (35). In addition, the amino terminus of p70 appears to be blocked. Acetylated methionine residues are generally not followed by serine (35, 36), while an amino-terminal serine residue is frequently acetylated (37), providing further indirect evidence that the amino-terminal residue *in vivo* may be serine rather than methionine.

Analysis of the predicted p70 amino acid sequence demonstrated two regions of possible α-helical secondary structure (Fig. 7) containing periodic repeats of either leucine and serine (residues 215–243) or leucine alone (residues 483–504) (Figs. 4 and 6). The Leu-Ser repeat region of p70 displays a possibly significant sequence similarity with a region of the v-*myc* and c-*myc* proteins that is essential for transformation (38), and which contains a leucine repeat with identical periodicity. While the functional significance of this similarity is difficult to assess at present, it is notable that two cellular differentiation factors, the MyoD1 protein (39) and the T4 achaetescute protein of *Drosophila* (40), also display comparable similarities with this region of *myc*.

The Leu- and Leu-Ser repeat regions of p70 are similar to leucine repeat regions found in a number of oncogene products and transcription factors (34). Many of these proteins contain a region rich in basic amino acids immediately adjacent to the leucine repeat, The Leu-Ser repeat of p70 is adjacent to a strongly basic region (Fig. 7) and the leucine repeat to a less strongly basic region (residues 461–482). In the model proposed by Landschulz *et al.* (34), the periodic repeat of leucine residues is thought to interdigitate with a similar domain of a second protein, juxtaposing the basic amino acids of the two proteins in a manner suitable for sequence-specific recognition of DNA. It remains to be determined whether either the

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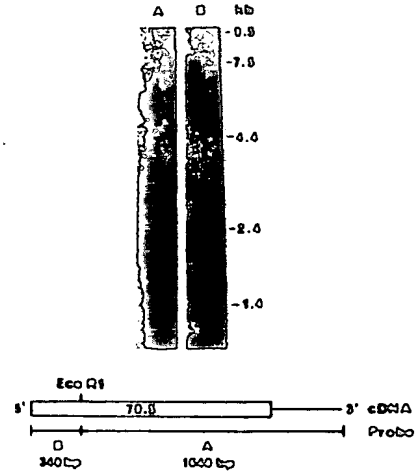

FIG. 5. RNA blots of K562 poly(A)$^+$. Poly(A)$^+$ RNA (13.2 μg/lane) was analyzed on a 1% agarose/formaldehyde gel and transferred to nitrocellulose. Blots were baked, prehybridized, and hybridized with ^{32}P-labeled *Eco*RI fragments of clone 70.5: *A* = ~1640 bp 3′ fragment; *B* = ~340 bp 5′ fragment. Both fragments hybridized with a RNA species of ~2.4 kb. Positions of RNA standards (Bethesda Research Laboratories, Gaithersburg, MD) are indicated.

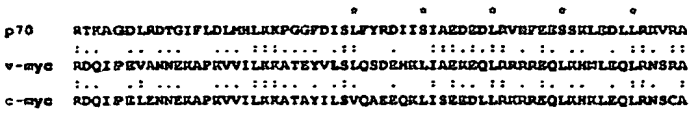

FIG. 6. Amino acid sequence similarity between p70, v-*myc*, and c-*myc*. The deduced amino acid sequence of p70 (residues 187–248) was aligned to maximize similarity with the amino acid sequences of v-*myc* (avian myelocytomatosis virus) (49), residues 361–422, and human c-*myc* (50), residues 399–460. This region of similarity coincides with the proposed "leucine zipper" domain of the *myc* proteins (34). Positions of the periodic repeats of leucine and serine (p70) or leucine alone (v-*myc* and c-*myc*) are indicated by °.

FIG. 7. Predicted secondary structures of similar regions of p70, v-*myc*, and c-*myc*. *A* denotes helix-permissive structure, *B* denotes β-sheet, and *T* denotes turn, as predicted by the program of Chou and Fasman (26). Positions of periodic repeats of leucine and serine (p70) or leucine alone (v-*myc* and c-*myc*) are indicated (°). Basic residues in a 22-amino acid region immediately following the leucine-serine repeat of p70 are indicated by *x*.

leucine repeat or the Leu-Ser repeat can participate in the formation of this hypothetical structure. In particular, we cannot be certain that a polar amino acid such as serine would be compatible with the interdigitation postulated by the Landschulz model. The sequence similarity of p70 with the leucine zipper region of *myc*, the α-helical secondary structure predicted for this region (Fig. 7), and the adjacent 22-residue basic domain may provide indirect evidence supporting this possibility. Clearly, however, further experimental evidence will be necessary to assess the functional significance, if any, of this region. If either of these repeats is involved in the formation of a leucine zipper, then the Landschulz model would predict the existence of a similar region(s) in the p80 protein. This prediction will be readily testable when the sequence of p80 is available.

The predicted amino acid sequence of p70 also contains two regions rich in acidic residues (61 residues, 31% Glu + Asp, and 19 residues, 58% Glu + Asp, see Fig. 4). These acidic regions are comparable in length and charge to the acidic domains found in GCN4 (60 amino acids, 30% Glu + Asp) (41), and GAL4 (29 residues, 31% Glu + Asp, and 20 residues, 35% Glu + Asp) (42) that are thought to play a critical role in transcriptional activation (41–43). In addition, the high frequency of serine residues in the 61-amino acid acidic domain raises the possibility that the negative charge of this region might be increased by phosphorylation. Since the acidity of an "acid blob" appears to correlate with its transcriptional potency (44), phosphorylation of this region, if it occurs, might have functional significance. Thus, the structure of p70 resembles that of GCN4 and *myc* proteins not only in containing one or more possible leucine zipper domains (34, 41), but also in containing an anionic region (41, 45). Based on the existence of both a possible DNA-binding domain(s) and a potential transcriptional activator domain (43), it is tempting to speculate that p70 might have a role in transcription. Alternatively, the structure of p70 might be consistent with a role in DNA repair (4) or replication. These possibilities are not mutually exclusive, since recent studies indicate that certain transcriptional activators may be components of eukaryotic origins of DNA replication (46, 47).

The present studies demonstrate the existence of a major autoantigenic epitope or epitopes on the carboxyl-terminal 190 amino acids of p70 (Fig. 3, *70.77*), a region containing the leucine repeat region of p70 (Fig. 4). We have previously found that autoantibodies in certain autoimmune sera inhibit the binding of p70/p80 to DNA, and conversely, that binding of DNA to p70/p80 partially inhibits autoantibody binding in some cases (2). Thus, at least one of the regions predicted to have a possible role in DNA binding may also be an important autoepitope. Recent studies from our laboratory suggest that the majority of autoantibodies to p70 in most sera from patients with SLE are reactive with this region.[3,4]

The observation that antibodies eluted from the 70.34 fusion protein were specific for p70, and displayed no cross-reactivity with p80 suggests that the carboxyl-terminal 239 residues of p70 may not have extensive homology with p80, an interpretation that is also supported by the observation that p70 cDNA hybridized with a single poly(A)$^+$ RNA (Fig. 5). It seems unlikely, therefore, that p70 and p80 are derived from a single gene by an alternative splicing mechanism. The possibility that p70 is derived from proteolytic cleavage of p80 is also highly unlikely. The immunologic cross-reactivity of p70 and p80 previously reported (6) may therefore reflect a relatively short region of p80 amino acid sequence similarity, possibly near the amino terminus of p70. We have been unable to test this possibility due to difficulties obtaining fusion proteins containing the amino-terminal 115 amino acids of p70. Although clone 70.5 contains these residues and was obtained by antibody screening, only trace amounts of fusion protein were produced by *E. coli* Y1089 lysogenized by this clone. Furthermore, we have been unable to express this region in a variety of plasmid expression vectors.[4] The difficulty in expressing this region might relate to amino acid

[4] W. H. Reeves and Z. M. Sthoeger, unpublished observations.

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sequences analogous to those that target certain proteins for rapid degradation in eukaryotic cells (48), or to low levels of synthesis and/or a high rate of degradation of the mRNA. Direct comparison of the sequence of p70 with that of p80, when available, may be necessary to localize the region(s) of immunologic similarity (6) between the two proteins. How autoimmunity to the p70 antigen develops, why it is closely linked to autoimmunity to p80, and whether the function of p70/p80 is related to the development of autoimmunity to the complex remain unanswered questions. The availability of the cloned autoantigens may be valuable in addressing these issues.

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1: NM_001469. Reports Homo sapiens X-ra...[gi:51093847] Links
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LOCUS       NM_001469                2156 bp    mRNA    linear   PRI 17-SEP-2006
DEFINITION  Homo sapiens X-ray repair complementing defective repair in Chinese
            hamster cells 6 (Ku autoantigen, 70kDa) (XRCC6), mRNA.
ACCESSION   NM_001469
VERSION     NM_001469.3  GI:51093847
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
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REFERENCE   3  (bases 1 to 2156)
  AUTHORS   Zhu,P., Zhang,D., Chowdhury,D., Martinvalet,D., Keefe,D., Shi,L.
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TITLE Cloning and characterization of a cDNA that encodes a 70-kDa novel human thyroid autoantigen
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PUBMED 2917966
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from CD683757.1, AK055786.1, BC018259.2 and BC012154.2.
On Aug 10, 2004 this sequence version replaced gi:20070134.

Summary: The p70/p80 autoantigen is a nuclear complex consisting of two subunits with molecular masses of approximately 70 and 80 kDa. The complex functions as a single-stranded DNA-dependent ATP-dependent helicase. The complex may be involved in the repair of nonhomologous DNA ends such as that required for double-strand break repair, transposition, and V(D)J recombination. High levels of autoantibodies to p70 and p80 have been found in some patients with systemic lupus erythematosus.
COMPLETENESS: complete on the 3' end.

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